

provide valuable insights into its mode of action. The molecular framework of DFBP resembles that of levosimendan, thus it was chosen to mimic levosimendan to establish how the cTnC-cTnI binding equilibrium is modulated. We have utilized 2D {¹H, ¹⁵N} HSQC and 2D {¹H, ¹³C} HSQC NMR spectroscopy to examine the binding of DFBP to cTnTc•Ca²⁺ in the absence and presence of cTnI144-163 and of cTnI144-163 to cTnTc•Ca²⁺ in the absence and presence of DFBP. The results show that DFBP and cTnI144-163 bind cTnTc•Ca²⁺ concurrently and the affinity of DFBP for cTnTc•Ca²⁺ is increased ~5-10-fold by cTnI144-163. We are in the process of determining the NMR solution structure of cTnTc•Ca²⁺•cTnI144-163•DFBP. This structure will contribute to the understanding of the mechanism of action of levosimendan in the therapy of heart disease. It will also provide a structural basis for the design of Ca²⁺-sensitizing drugs in general.

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Decreased Fatigue Tolerance In Diaphragm Muscle Of Slow Troponin T Knockdown Mice

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The loss of slow skeletal muscle troponin T (TnT) results in a severe type of nemaline myopathy in the Amish (ANM). The genes encoding TnT and troponin I (TnI) are closely linked in pairs in which the 5'-enhancer region of the slow TnT gene overlaps with the cardiac TnI gene. In a mouse line with the entire cardiac TnI gene deleted, a partial destruction of the slow TnT gene promoter produces a knockdown effect. By crossing with transgenic mouse lines that over-express a core structure of cardiac TnI (cTnI-ND) under the control of cloned alpha-MHC promoter, we rescued the postnatal lethality of the cardiac TnI gene-deleted mice with no detrimental cardiac phenotypes or leaking expression in non-cardiac tissues. The double transgenic mice exhibited decreased expression of slow TnT mRNA and protein in adult diaphragm muscle. Functional analysis of isolated muscle strips showed that the slow TnT deficient (sTnT-KD) diaphragm had significantly decreased fatigue tolerance evident by the faster decrease in force and slower rate of recovery as compared with that in wild type controls. As a consequence of slow TnT deficiency, the sTnT-KD diaphragm muscle contained a higher proportion of fast TnT, decreased slow TnI with increased fast TnI, and decreased type I myosin with increased type II myosin. Consistent with the switch toward fast myofilament contents, the sTnT-KD diaphragm muscle produced higher specific tension in twitch and tetanic contractions as well as shorter time to develop peak tension in twitch contractions. The decreased fatigue tolerance of sTnT-KD diaphragm muscle explains the terminal respiratory failure seen in virtually all ANM patients and this double transgenic mouse model provides a useful experimental system to study the pathogenesis and treatment of ANM.

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Troponin Isoforms and Stretch-activation of Insect Flight Muscle

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Oscillatory contraction of insect indirect flight muscle (IFM) is activated by sinusoidal length changes. Work done by oscillating fibres is measured from the area of loops on a length-tension plot. At [Ca²⁺] above 10 µM, progressively less oscillatory work is produced because fibres contract isometrically and are unable to relax fully after each cycle of oscillation. Periodic stretches during oscillations activate fibres through the action of TnC F1, which binds one Ca²⁺ in the C-lobe. Activation of isometric contraction by Ca²⁺ acts through F2, which binds Ca²⁺ in both N- and C-lobes. Lethocerus IFM fibres substituted with F1 gave oscillatory work, which did not decline at high [Ca²⁺], while fibres substituted with F2 produced more isometric tension as [Ca²⁺] was increased. Varying proportions of F1 and F2 gave maximal work with an F1:F2 ratio of 100:1, which is higher than the in vivo ratio of 7:1. The structure of F1, and the interaction with TnI, were determined by NMR. The N-lobe of F1 is in the closed conformation in apo and Ca²⁺-bound forms and does not bind TnI. Unexpectedly, the C-lobe is open in both states, and binds the N-terminal domain of TnI independently of Ca²⁺. The affinity of F1 and F2 for a complex containing tropomyosin, TnT and TnH (Lethocerus TnI) were measured by isothermal calorimetry in the presence of Ca²⁺. The affinities of F1 and F2 for the complex were 5.4 µM and 65 nM respectively. This difference is likely to be due to a single TnI binding site on F1 and two sites on F2. Stretch may be sensed by an extended C-terminal domain of TnH, and transmitted to the C-lobe of F1, resulting in a change in the interaction of the TnI inhibitory domain and actin.

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Tracking of Qdot Conjugated Titin Antibodies in Single Myofibril Stretch Experiments Reveals Ig-domain Unfolding at Physiological Sarcomere Lengths

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The mechanical characteristics of titin in muscle sarcomeres were previously studied by us in single myofibril stretch experiments, where the extensibility of I-band titin segments was usually measured under static conditions. Here we investigated the behavior of I-band titin during and after stretch of single rabbit psoas myofibrils in real-time. The focus was on titin's proximal Ig-domain region, whose stretch dynamics were analyzed by labeling the myofibrils specifically in the N2A-titin domain using antibody-conjugated quantum dots, which stained the periphery of the myofibril but did not enter the myofilament lattice. Qdot labels were tracked to obtain the stretch-dependent change in epitope distance (across Z-disc) and sarcomere length (SL) over time. In contrast to what was expected from the current titin extensibility model, at sarcomere lengths of 2.5 and 3.8 µm, titin's proximal Ig-domain region elongated continuously, in proportion to the half I-band length. Already at ~2.6 µm SL the proximal Ig-segment length exceeded the value expected if all Ig-domains remain folded. Our results suggest that Ig-domains unfold in parallel with PEVK-titin extension at physiological sarcomere lengths and under relatively low forces. By reducing the antibody-Qdot concentration, we succeeded in observing titin Ig-domain dynamics in myofibrils at the single-molecule level.

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Constitutive Phosphorylation of Cardiac Myosin Binding Protein-C Increases the Probability of Myosin Cross-bridge Interaction with Actin

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Protein kinase A-mediated (PKA) phosphorylation of cardiac myosin binding protein-C (cMyBP-C) accelerates the kinetics of cross-bridge cycling and appears to relieve the tether-like constraint of myosin heads imposed by cMyBP-C (Colson *et al.*, 2008, *Circ Res.*, 103:244-251). We favor a mechanism in which phosphorylation of the 3 PKA sites in cMyBP-C modulates cross-bridge kinetics by regulating the proximity and interaction of myosin with actin. To test this idea, we used synchrotron low-angle x-ray diffraction and mechanical measurements in skinned myocardium isolated from a mouse model with phosphomimetic substitutions in cMyBP-C, i.e., the CTSD mouse. The substitutions were introduced by transgenic expression of cMyBP-C with Ser-to-Asp mutations on a cMyBP-C null background. Western blots showed that expression of CTSD cMyBP-C was 85% of wild-type (WT), and the heart weight to body weight ratio was similar (5.2 ± 0.2 mg/g) in CTSD and WT mice. Expression of WT cMyBP-C on the knockout background served as control (i.e., the CTWT mouse). Skinned myocardium from CTSD and CTWT mice exhibited similar maximum active forces (mN/mm²: 17.7 ± 3.7 vs 13.2 ± 2.9), Ca²⁺-sensitivities of force (pCa₅₀: 5.55 ± 0.03 vs 5.58 ± 0.04), and maximum rates of force development (*k_{tr}*, sec⁻¹: 20.2 ± 1.7 vs 22.5 ± 1.9; *k_{dr}*, sec⁻¹: 37.6 ± 3.7 vs 43.2 ± 2.3). *I*₁₁₀/*I*₁₀ intensity ratios and *d*₁₀ lattice spacings determined from equatorial reflections from CTSD and CTWT myocardium were used to determine the effect of constitutive cMyBP-C phosphorylation on the distribution of cross-bridge mass between the thick and thin filaments and on interfilament lattice spacing. The results suggest that interactions between cMyBP-C and the S2 domain of myosin heavy chain are dynamically regulated by phosphorylations in the cMyBP-C motif. (AHA-predoctoral fellowship (BAC); NIH-HL-R01-82900)

1196-Pos Board B40

Obscurin Interacts with a Novel Isoform of Myosin Binding Protein C-Slow to Regulate the Assembly of Thick Filaments

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Obscurin is a multidomain protein composed of adhesion and signaling domains that plays key roles in the organization of contractile and membrane structures in striated muscles. We used adenoviral-mediated gene transfer to overexpress its extreme NH2-terminus in developing myofibers, followed by immunofluorescence and ultrastructural methods to study its effects in sarcomerogenesis. We found that overexpression of obscurin's second immunoglobulin domain (Ig2) inhibits the assembly of A- and M-bands, but not Z-disks and